Reactivated Response of Blink Reflex in the Cat

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Abstract Three reflex responses in the orbicularis oculi muscles were observed by electrical stimulation of the trigeminal nerve in lightly anesthetized or conscious cats. The third response, following the first and second responses, was concluded as being a "reactivated response" of the blink reflex as it was set up by the trigeminal afferent input evoked by the palpebral movement of the preceding blink. The reasons for this were as follows: 1) the third response disappeared on administration of muscle relaxants and 2) was enhanced by touching the palpebral hair; 3) the palpebral movement activated the afferent discharges of the trigeminal exteroceptors which were strong enough to yield the reflex responses and 4) the latency of the first reflex response by the palpebral movement corresponded to the time lag between the preceding large reflex response and the third response; 5) the third response was hardly suppressed at all until the first response disappeared over a period of maximum reflex suppression following the conditioned stimulation of the trigeminal nerve. *Key Words:* blink reflex, facial reflex, trigeminal nerve, brain stem, cat.

Tapping the facial skin or electrical stimulation of the trigeminal nerve elicits the blink reflex responses in human and animal orbicularis oculi muscles (EKBOM *et al.*, 1952; KUGELBERG, 1952; TOKUNAGA *et al.*, 1958). Particular attention was given to the two successive reflex responses of the orbicularis oculi muscles. The input source of the blink reflex was found to be the trigeminal nerve (LINDQUIST and MARTENSSON, 1970; PENDER and DELWIDE, 1973; SHAHANI and YOUNG, 1973), although some afferent fibers in the facial nerve might mediate the facial reflex (RUSHWORTH, 1962; LINDQUIST and MARTENSSON, 1970). However, little attention is paid, regarding the analysis of the blink reflex, to the fact that the third response often appears after the two successive responses in the orbicularis oculi muscles to electrical stimulation of the trigeminal nerve of the cat.

This paper describes the third response in the orbicularis oculi muscles appearing after the two successive responses of the blink reflex in the cat and reveals

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that the third response is a "reactivated response" of the blink reflex elicited recurrently by the palpebral movement of the preceding blink.

METHODS

Experiments were performed on 22 adult cats weighing 2 to 4.5 kg on a stereotaxic apparatus. Anesthesia was induced by ether and maintained by injections of chloralose (Tokyo Kasei, 50–150 mg/kg) through a catheter placed in the cephalic vein. Artificial respiration was used in preparations treated with dantrolene sodium (Yamanouchi) or gallamine triethiodide (Teikoku) to achieve muscle relaxation. Experiments were started at least 2 hr after stopping ether anesthesia.

The infraorbital or supraorbital nerve of the trigeminal nerve was stimulated electrically using a stimulator (Nihon Kohden, MES-3). Pulses of 0.3 msec duration were applied every 5 sec through the bipolar silver electrodes of 0.5 mm diameter. Action potentials in the afferent and efferent nerves were led from the supraorbital nerve and the palpebral branch of the facial nerve (palpebral nerve) respectively, through similar electrodes used for the stimulation and displayed on an oscilloscope (Nihon Kohden, VC-7). Afferent discharges of the supraorbital nerve were recorded by adding 50 successive responses with a data-processing computer (Nihon Kohden, ATAC-2300). Electromyograms (EMGs) of the orbicularis oculi muscles were recorded by enamel wire electrodes of 0.03 mm diameter with tips about 1 mm long stripped and coated with AgCl. The isotonic tension of the palpebral muscle was recorded by a tension transducer specially constructed so as not to disturb the palpebral movement.

In five preparations, wire electrodes were implanted chronically under ether anesthesia in the orbicularis oculi muscles and in the supraorbital nerve to record the blink reflex responses in the conscious cats. The experiments were performed at least one week after the operation.

RESULTS

1) Three successive responses in the blink reflex

Three successive responses were observed by electrical stimulation of the infraorbital or supraorbital nerve in lightly anesthetized cats (chloralose 60 mg/kg, i.v.). In order to demonstrate the thresholds and latencies of these responses we compared the EMGs for different stimulus intensities. Because of the surface stimulation of the nerve, we could not establish quantitative measurement of the absolute intensity, but qualitative comparison of these three responses was possible using the same electrode and preparation.

As shown in Fig. 1, the first and second responses were observed 9.0 and 18.0 msec respectively after stimulation of the infraorbital nerve when the stimulus

Fig. 1. Change of blink reflex responses in the orbicularis oculi muscles on increasing the stimulus strength of the infraorbital nerve in lightly anesthetized cat. Note the third response (arrow) after two successive responses occurred when the stimulus intensity increased. Calibration: 10 msec, 1 mV.

intensity used was 0.4 V (Fig. 1, top trace). The latency of the first response as well as the second response became shorter with increasing stimulus intensity. As the stimulus strength exceeded 1.2 V, the third response occurred 27.0 msec after stimulation (Fig. 1, third trace, arrow). When the stimulus increased further (2.0 V, Fig. 1, bottom trace), the third response continued over 15 msec, and frequently it appeared in two separated responses. In four out of 17 experiments the group of action potentials appeared contralaterally (Fig. 1, right column, bottom trace) and the latency was of approximately the same order as the third response observed ipsilaterally. In 17 cats, the latency of the first and second responses was 5.8–8.0 (mean 7.2) and 12.4–17.0 (mean 14.3) msec respectively, whereas the latency of the third response varied over a wider range of 22.0–34.0 (mean 28.2) msec.

The third response was also observed ipsilaterally in the conscious and unrestrained cats following two successive responses, but no responses were observed contralaterally. In the example shown in Fig. 2, the first response occurred at 6.5 msec, the second at 16.0 msec and the third at 27.0 msec after stimulation of the supraorbital nerve. These latencies were of about the same order as in those anesthetized animal. The above findings were confirmed in 4 other examples.

These experiments, shown in Figs. 1 and 2, indicate that the third response occurred only when the stimulus intensity increased. That is, the third response was evoked by stronger stimulation than that for activating the first and second responses.

Fig. 2. Blink reflex responses in the orbicularis oculi muscles evoked by stimulation of the supraorbital nerve in conscious and unrestrained cats with chronically embedded stimulating and recording electrodes. Note the third response (arrow) occurred following two successive responses at the ipsilateral side but neither response was seen at the contralateral side. (Irregular basal lines at the left end of each trace are artificial noise on recording.) Calibration: 10 msec, 1 mV.

2) Characteristics of the third response

To gain further knowledge on the characteristics of the third response, various conditions which depressed or enhanced the third response were studied in lightly anesthetized cats (chloralose 50–60 mg/kg, i.v.). The results are summarized in Fig. 3.

The level of anesthesia had an effect on the third response (Fig. 3-1). The third response (arrow) disappeared within 20 min of an additional application of chloralose (total dose 100–150 mg/kg). However, it reappeared as the animal began to awake from anesthesia. The experiments illustrated in Figs. 3-2 and 3-3 show the effect of muscle relaxants such as gallamine triethiodide and dantrolene sodium. The third response disappeared within 10 min of the administration of the dantrolene sodium (4 mg/kg, i.v., Fig. 3-2) which reduced the twitch contraction of the skeletal muscle by about 60–70% at the peripheral site without affecting the neuromuscular junction (*cf.* HONKOMP *et al.*, 1970; TAMAI *et al.*, 1973). Similarly the third response disappeared when gallamine triethiodide (4 mg/kg, i.v.) was injected (Fig. 3-3). In this experiment, the reflex responses were recorded from the palpebral nerve, as the EMGs of the orbicularis oculi muscles did not appeared by the effect of the gallamine triethiodide on the neuromuscular junction. The experiments illustrated in Fig. 3-4 show that the third response was enhanced when the palpebral hairs were touched with a small brush during stimulation of the supraorbital nerve. These results indicate that the palpebral movement may be important for the appearance of the third response.

When the supraorbital nerve was stimulated twice at various intervals, the reflex responses due to succeeding stimulations (test stimulus) were affected by preceding stimulations (conditioned stimulus). That is, the second response due

Fig. 3. Disappearance or enhancement of the third response under various conditions (see text). Calibration: 10 msec, 0.5 mV.

to the test stimulus was suppressed when the intervals between the conditioned and test stimuli were set at 5 to 30 msec, but the first response was facilitated. Both responses, however, were suppressed completely when the interval of the two stimuli was about 50 msec and recovered gradually over a 80 msec interval (*cf.* LINDQUIST, 1972). Given this situation, the question whether the third response was suppressed or not was examined.

The control responses to the conditioned stimulus and test stimuli are shown as the two upper traces in Fig. 4. No response to the conditioned stimulus (S1, left uppermost trace) was recorded as a subthreshold stimulation was selected for the conditioned stimulus to escape the duplication of the reflex responses. On the other hand, three typical successive responses to the test stimulus (S2, left second trace) were observed. When the test stimulus was applied 10, 16, 25, and 40 msec following the conditioned stimulus, the second response was suppressed but the third response persisted (see arrows). However, the third response was suppressed when the first response was suppressed by a period of maximum reflex suppression after the conditioned stimulus (middle trace in right-hand column). The third response appeared again with the first and second responses as the suppressive effect of the conditioned stimulus became extinct (lower two traces in right-hand column). This indicates that the effect of the conditioned stimulus on the third response is similar to that on the first response.

Fig. 4. Effect of the conditioned stimulus on the third response. The supraorbital nerve was stimulated twice with various intervals (number beside each trace). The two traces on the upper left show the control responses to the conditioned (S1) and the test (S2) stimuli, respectively. No response to the conditioned stimulus was recorded as a sub-threshold stimulation was selected for the conditioned stimulus to escape the duplication of the three reflex responses. Small S1 and S2 under traces show the relative period of the conditioned and test stimulations. In the right-hand column, all records by the conditioned stimulus (S1) were omitted. Note the persistent third response (arrows) following the facilitated first response despite the suppression of the second response at 10, 16, 25, and 40 msec intervals of the conditioned and test stimuli. Calibration: 10 msec, 1 mV.

3) Activation of the trigeminal exteroceptors and reflex responses by palpebral movement

The afferent discharges in the supraorbital nerve were recorded with palpebral movements to investigate whether the contraction of the orbicularis oculi muscles activated the trigeminal exteroceptors (Fig. 5). The palpebral nerve was cut and the peripheral cut-end (S in inset) was stimulated to produce the contraction of the orbicularis oculi muscles (B in inset) and the central cut-end (D in inset) was used for recording of the reflex responses. The afferent discharges were recorded from the supraorbital nerve (C in inset) by adding 50 successive responses with the computer.

As shown in Fig. 5, afferent discharges in the supraorbital nerve (trace C) were observed during palpebral contraction (trace B) on stimulation of the palpebral nerve. The discharges of the trigeminal exteroceptors were larger at the initiation of the palpebral movement (arrow in trace C) and lasted during the palpebral movement while decreasing in amplitude. The large initial deflection on the trace C (before arrow) was an artifact reflecting the large action potential of the orbicularis oculi muscles. Following the trigeminal afferent responses,

Fig. 5. Afferent responses in the supraorbital nerve and their reflex responses in the palpebral nerve evoked by palpebral movement. A: EMG of the orbicularis oculi muscles. B: contraction curve of the orbicularis oculi muscles elicited by stimulation of the peripheral cut-end of the palpebral nerve (S in inset). C: afferent discharges in the supraorbital nerve obtained by computer averages of 50 responses. The initial large deflection on the trace C (before arrow) was an artifact reflected in the EMG of the orbicularis oculi muscles. D: the reflex responses in the central cut-end of the palpebral branch. E and F: three responses in the orbicularis oculi muscles evoked by stimulation of the supraorbital nerve before cutting the palpebral nerve. Note that the time lag between the large EMG and the third response in traces E and F (horizontal bars in traces E and F) corresponded to the time lag between the EMGs in trace A and the first reflex response in trace D (horizontal bar under trace D). Calibration: 10 msec; A, E, F, 1 mV; B, 0.2 g; C, 0.01 mV; D, 0.5 mV.

the reflex responses (trace D) appeared in the palpebral nerve and their latencies decreased with increasing amplitude of the palpebral movement.

The traces E and F in Fig. 5 show three responses of the blink reflex elicited by stimulation of the supraorbital nerve before cutting the palpebral nerve in the lightly anesthetized cat (chloralose 60 mg/kg, i.v.). The time lag between the large EMG and the third response in traces E and F (horizontal bars under traces E and F) corresponded to the time lag between the EMG in trace A and the first reflex response in trace D (horizontal bar under trace D). These results indicate that the third response in traces E and F is a "reactivated response" produced by the preceding large reflex response which is probably enough to evoke the trigem-

inal afferent volley by palpebral movement. In other words, the palpebral movement of the preceding blink reflex activates the trigeminal exteroceptors in the skin adjacent to the palpebra and their afferent impulses yield new blink reflex responses recurrently.

DISCUSSION

Two successive evoked EMGs of the orbicularis oculi in the blink reflex were first reported by KUGELBERG (1952) and coworkers by means of electromyographic analysis. These two responses are usually evoked in pairs by electrical stimulation of the trigeminal nerve and are respectively named the first and second, or early and late, responses. The first response is transmitted through an oligosynaptic reflex arc *via* the sensory trigeminal nucleus and the facial nucleus, whereas the second response is considered to have more neurons passing through the brain stem (LINDQUIST and MARTENSSON, 1970; HIRAOKA and SHIMAMURA, 1977). The first (early) and second (late) responses had latency ranges of 9–12 and 15–25 msec respectively on tapping the skin over the orbicularis oculi muscle (LINDQUIST and MARTENSSON, 1970) and of 7.5 ± 0.5 and 15.1 ± 1.4 msec on electrical stimulation of the cornea in the cat (HIRAOKA and SHIMAMURA, 1977). In the authors' experiments, the latency ranges of the two successive responses were 5.8–8.0 and 12.4–17.0 msec respectively on electrical stimulation of the infraorbital nerve. Therefore, the reflex response with a longer latency than that of the second response described above may be produced by a route other than that of the two successive responses.

It is not difficult to expect that palpebral movement activates the exteroceptors in the palpebral skin, as a slight touch of the palpebral hair is sufficient to elicit the blink reflex response in the cat. LINDQUIST and MARTENSSON (1970) suggested that the trigeminal exteroceptive afferents elicited by palpebral movement might contribute to the second reflex component. In the present experiments, it is revealed that the third response of long latency following the two successive responses was a "reactivated response" produced by the palpebral movement of the preceding blink reflex. That is, the palpebral movement activated the afferent discharges of the trigeminal exteroceptors which were strong enough to yield the reflex response, and the latency of the reflex response produced by the palpebral movement corresponded to the time lag between the large preceding response and the third response as shown in Fig. 5. Therefore, the third response diminished when the palpebral movement of the preceding blink was prevented by administration of muscle relaxants and was enhanced by touching the palpebral hair as described in Fig. 3, and behaved as the first response of the blink reflex in the experiment of double stimulation as seen in Fig. 4.

The reactivated response is easily identified when it appears after two successive responses but is confused with the second response of long latency when

the preceding two responses approach each other. A strict distinction between the second and reactivated responses is important for the analysis of the blink reflex since the reactivated response may have the same reflex pathway as that of the first response and is easily influenced by the conditions of the preparation such as degree of anesthesia, nerve stimulation and head fixation. The contralateral reflex response observed by strong stimulation of the infraorbital nerve in Fig. 1 (right bottom trace), for example, was not the second response appearing contralaterally such as seen in human blink reflex but the reactivated response evoked by the contralateral afferent input set up by the preceding facial reflex, because the contralateral response disappeared together with the ipsilateral reactivated response on administration of muscle relaxants.

The results resolve the tenacious question of whether the second response of the blink reflex in the cat is elicited ipsilaterally or bilaterally as reported by TOKUNAGA *et al.* (1958). In our experiments, two successive responses were observed only ipsilaterally in both anesthetized and conscious cats, except for the contralateral reactivated response. The second response reported by TOKUNAGA *et al.* (1958) seems to be the reactivated response described in this paper.

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